IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Kohei TOYOOKA, et al.

Serial No.: 10/534,414 Group Art Unit:1624

Filed: May 11, 2005 Examiner: Noble E. JARRELL

For: ISOINDOLINE DERIVATIVE

DECLARATION UNDER RULE 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Masakazu YOSHIMURA, citizen of Japan residing at Hyogo, Japan hereby declare and say that:

1. I am one of the joint inventors of the above identified application;

2. I graduated from Osaka University of Pharmaceutical Sciences, Japan in March, 1981;

3. I received a Doctor of Philosophy Degree in November, 2002 from the Graduate School of Osaka University;

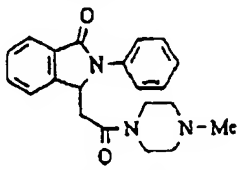
4. Since April, 1981 up to this time, I have been an employee of Maruishi Pharmaceutical Co., Ltd., the assignee of the above-identified application, and have been engaged in the research and development work in the field of pharmacology;

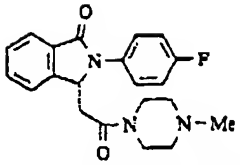
5. I am a member of the Japanese Pharmacological Society, Japanese Association for the Study of Pain, and the Japan Neuroscience Society;

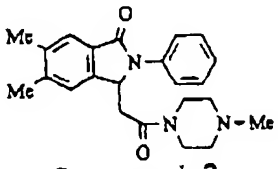
6. I have read the Office Action mailed on November 23, 2007 and the references cited therein and am familiar with the subject matter thereof;

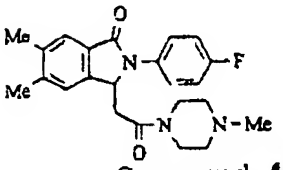
7. To demonstrate the unexpected superiority of the present invention over Hiraga et al (US4590189), I set forth below comparative experimentation that was conducted under my supervision:

Compounds Tested:


Compound **1**


Compound **2**


Compound **3**


Compound **4**

Chemical structure of Compoundd **1-4**

Method:

Animals and Compounds

Male ICR mice (4 weeks old) were used. Mice were maintained in a climate-controlled room on a 12-h light-dark cycle and were allowed food and water *ad libitum*.. Each compound was prepared as the hydrochloride salt and was dissolved in saline. The rate and volume of injection through the lateral tail vein was 0.1 ml/10 seconds and 0.1 ml/10 g body weight, respectively.

Determination of loss of righting reflex

The loss of righting reflex (LRR) was examined using 2 or 3 of male ICR mice. A selected dose of each compound was injected intravenously. After each injection, mice were placed on their backs. If the animal remained on its back, it was deemed to have lost its righting reflex. Onset of LRR, and time the animals regained the righting reflex (GRR) were measured. Sleep time was obtained as LRR-GRR.

Determination of HD_{50}

The hypnotic dose (HD_{50}) of compound **3** and **4** were obtained using male ICR mice. Six mice per dose were used. A selected dose of each compound was injected intravenously. After each injection, mice were placed on their backs to test for loss of righting reflex (LRR) as an index of hypnotic activity. From the percentage of mice in each group showing LRR for 30 seconds or longer, a probit analysis was used to calculate 50% hypnotic dose (HD_{50}).

Results:

Determination of loss of righting reflex

Compounds **1** and **2** did not show LRR even at the dosage of 40 mg/kg. The sleep time of compounds **1** and **2** were 0 second. On the other hand, Compounds **3** and **4** showed the LRR at the doses of 10 mg/kg and 15 mg/kg, respectively. The mean sleep time of Compound **3** and **4** were 330 seconds and 95 seconds, respectively.

Determination of HD_{50}

Because Compound **1** and **2** did not show LRR, HD_{50} was not measured. The HD_{50} of Compounds **3** and **4** were 9.37 mg/kg and 14.72 mg/kg, respectively.

Discussion:

Compounds **1** and **2**, which are the compound similar to the compound of the invention but having H as the variable R_1, do not have anesthetic effect while the compounds **3** and **4**, which are comparative to Compounds **1** and **2** but have di-alkyl as variable R_1, have said effect. Accordingly, the compound of the invention have unexpected effect over the compounds disclosed in Hiraga et al and therefore.

8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This day of February 21, 2008

Masakazu Yoshimura

Masakazu YOSHIMURA